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Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean.

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ABSTRACT

Microplastics are widespread in the natural environment and present numerous ecological threats. While the ultimate fate of marine microplastics are not well known, it is hypothesized that the deep sea is the final sink for this anthropogenic contaminant. This study provides a quantification and characterisation of microplastic pollution ingested by benthic macroinvertebrates with different feeding modes (*Ophiomusium lymani*, *Hymenaster pellucidus* and *Colus jeffreysianus)* and in adjacent deep water > 2200 m, in the Rockall Trough, Northeast Atlantic Ocean. Despite the remote location, microplastic fibres were identified in deep-sea water at a concentration of 70.8 particles m^{-3}, comparable to that in surface waters. Of the invertebrates examined (n = 66), 48 % ingested microplastics with quantities enumerated comparable to coastal species. The number of ingested microplastics differed significantly between species and generalized linear modelling identified that the number of microplastics ingested for a given tissue mass was related to species and not organism feeding mode or the length or overall weight of the individual. Deep-sea microplastics were visually highly degraded with surface areas more than double that of pristine particles. The identification of synthetic polymers with densities greater and less than seawater along with comparable quantities to the upper ocean indicates processes of vertical re-distribution. This study presents the first snapshot of deep ocean microplastics and the quantification of microplastic pollution in the Rockall Trough. Additional sampling throughout the deep-sea is required to assess levels of microplastic pollution, vertical transportation and sequestration, which have the potential to impact the largest global ecosystem.

Capsule

41 Microplastics were identified in deep-sea benthic invertebrates and adjacent water > 2200 m deep
42 in the Rockall Trough with quantities comparable to surface concentrations.
43
44 **INTRODUCTION**

45 Plastic debris is a pervasive anthropogenic contaminant found extensively in the aquatic
46 environment worldwide (Cozar et al., 2014; Hammer et al., 2012). As a major source of marine
47 pollution, plastic debris meets ocean health index criteria and has been recognized as a global
48 threat, joining other marine stressors such as climate change, ocean acidification, overfishing and
49 habitat destruction (Amaral-Zettler et al., 2015; Halpern et al., 2012). The majority of plastic items
50 manufactured have single-use application (Thompson et al., 2009) and between 4.8 x 10^9 to 12.7
51 x 10^9 kg of plastic is estimated to have entered the ocean in 2010 alone (Jambeck et al., 2015); by
52 contrast an estimated 2.7 x 10^8 kg is afloat in surface waters (Eriksen et al., 2014). The progressive
53 fragmentation of plastic objects into ever smaller and more numerous pieces should lead to the
54 gradual increase of microplastics quantities (Andrady, 2011; Cozar et al., 2014; ter Halle et al.,
55 2016), however global budgeting identifies major discrepancies between the abundance of plastics
56 in surface waters, especially when considering microplastic particles (Cozar et al., 2014; Eriksen
57 et al., 2014).

58 Microplastics, defined here as particles 1 µm - 5 mm in diameter (Arthur et al., 2009) are
59 of particular environmental concern as they are a similar size to prey items and sediment grains
60 and are therefore bioavailable to a wide diversity of organisms. Ingestion is reported in numerous
61 species with documented impacts ranging from lethal to sub-lethal (Browne et al., 2008; Cole et
62 al., 2015; Murray and Cowie, 2011; Welden and Cowie, 2016; Wright et al., 2013a), and trophic
63 transfer of microplastics has been observed (Farrell and Nelson, 2013; Setälä et al., 2014).

Additionally, small particles have been shown to translocate within the bodies of crabs and mussels (Browne et al., 2008; Farrell and Nelson, 2013), consequently microplastics potentially have a greater toxological effect than larger plastic items. The high surface area to volume ratio means small particles have a greater area over which to absorb environmental contaminants; these may accumulate in the plastic, however the effect of plastic co-contaminants on biota is not yet clear (Koelmans, 2015).

The long-term fate and 'lifecycle' of microplastics in the marine environment is poorly understood. Distribution is influenced by abiotic (ocean currents, physical shearing, fragmentation and natural sinking (GESAMP, 2015)) and biotic factors (such as fouling (Fazey and Ryan, 2016), consumption and incorporation in faecal material (Cole et al., 2016) and settling detritus (Long et al., 2015)). These provide vertical transport pathways for microplastics from the sea surface to the benthos, thus it is hypothesized that microplastics are sequestered in the deep sea. There is a severe paucity of knowledge regarding microplastic pollution in the deep sea; however within the last few years microplastics have been documented in deep-sea sediments in regions of the Mediterranean Sea and the Atlantic, Pacific and Indian Oceans (Fischer et al., 2015; Van Cauwenberghe et al., 2013; Woodall et al., 2014), and more recently isolated from deep-sea benthic invertebrates (Taylor et al., 2016).

This study aims to provide a thorough assessment and quantification of microplastic ingestion by deep-sea benthic invertebrates displaying different feeding modes and presents the first quantification of microplastic pollution in deep-sea water. To test the hypothesis that microplastics are present at a deep-sea site in the Rockall Trough, Northeast Atlantic Ocean, benthic fauna and water samples were collected from a depth > 2200 m. Samples were analysed

to i) determine whether microplastics occur in this remote deep-sea location and ii) characterise and quantify the microplastics present.

MATERIALS AND METHODS

Sampling location

The Rockall Trough is situated to the west of Scotland, UK. The monitoring site, 'Gage Station M', is located in the Rockall Trough (57.300°N, -10.383°W) near the foot of Anton Dohrn seamount at a depth of 2200 m (Figure 1). During the 2016 research cruise DY052 aboard *R.R.S. Discovery*, four epibenthic sled tows and one Conductivity, Temperature, Depth (CTD) cast for deep-sea water were undertaken.

Field methods

On-board quality assurance/quality control (QA/QC)

QA/QC procedures were designed and employed at all stages to reduce the potential for sample contamination. Standard non-plastic equipment such as metal and glass were used as much as possible; all equipment was cleaned thoroughly by wiping with 70 % ethanol on non-shedding paper three times prior to use. Ships water supplies were fitted with a mesh cartridge filter to remove contaminants, these were tested for efficiency prior- and post-sampling by running water through an 80 µm filter for two hours and examining these under the microscope. Prior to work commencing and between each sled haul the deck was washed down with the ship's fire hose. The number of people working on samples was kept to a minimum. The same personal protective equipment was worn for the duration of sampling and stored separately. Sample fibres from

clothing, along with any potential contaminants from the research vessel such as ropes, piping, mesh screens etc were taken to be analysed alongside the deep-sea samples.

Deep-sea benthic sampling

Two Woods Hole Oceanographic Institution-pattern epibenthic sleds, rigged with main and extension nets of mesh size 0.5 mm were used to obtain samples following historical methods. The sleds were deployed individually down to the seafloor with the doors open and trawled along the seabed for ~60 minutes before the sled doors closed by a pre-set timer mechanism and the net hauled slowly to the surface. Once on-board the net was opened and material was emptied into lidded plastic buckets, before being washed over stacked sieves of mesh sizes 4 mm, 0.5 mm and 0.42 mm. Macrofauna retained on the 4 mm sieve were individually wrapped in aluminium foil, placed in lidded buckets separated by taxonomic groups and frozen at -20°C to be utilized in this study.

Deep-sea water sampling

Two-hundred and forty litres of water were collected using a Sea-Bird 24-way CTD system with stainless steel frame. All 24 niskin bottles were fired 7 m from the seabed at a depth of 2227 m. On deck prior to sampling, the spigot of each niskin bottle was cleaned by rinsing it thoroughly with deionised water and all water filters and hosing were examined carefully to ensure they were free from contaminants. Niskin bottles were systematically sampled by running the entire volume of water through an 80 µm mesh filter until water flow completely ceased. All sampling was carried out by one individual who remained downwind of the filter throughout. Upon completion,

130 filters were placed in a clean petri dish, sealed with tape and labelled for analysis once back in the
131 laboratory.
132
133 **Laboratory methods**
134 **Laboratory QA/QC**
135 Samples were prepared and analysed in a separate small laboratory only used by the scientist
136 carrying out the analysis. Air vents were sealed and the door remained closed for the duration of
137 the experiment to reduce air-borne contamination sources. The work benches were cleaned with
138 70 % ethanol on non-shredding paper and allowed to dry fully; this was repeated three times prior
139 to commencing work. Standard non–plastic equipment i.e. glass and metal, were used wherever
140 possible and consumables were used directly from sterile packaging. All apparatus was washed
141 with deionised water prior to use and equipment was inspected under a dissecting microscope. The
142 samples were kept covered to minimize exposure risk. Natural fibre clothes were worn under a
143 clean 100 % cotton laboratory coat, these clothes were stored in the laboratory to avoid contact
144 with external synthetic fibres.
145 Background laboratory contamination was assessed in two ways based on (Courtene-Jones
146 et al., 2017). Dampened filter paper (30 mm diameter, Whatman No. 1) was placed into a clean
147 petri dish and left exposed for the duration of the experiment to monitor air-borne fibres, these
148 were then sealed and labelled for further analysis. Tape lift screening (TLS) was employed to test
149 for surface microplastics; after the benches had been cleaned, a 5 cm^2 piece of adhesive tape was
150 cut and placed on the bench surface in three random locations before being placed on an acetate
151 sheet and examined under a microscope, this process was carried out before and after laboratory
152 procedures. Samples of putative contaminants, such as the sterile packaging, adhesive tape and

153 acetate sheet used for TLS, natural fibre clothing and filter paper used were taken to be analysed
154 alongside the deep sea samples.
155
156 **Inspection of deep-sea water filters**
157 The 80 μm mesh filters were transferred to individual lidded glass petri dishes. The gauze were
158 systematically and thoroughly examined under a dissecting microscope (Wild M5); any potential
159 microplastics were removed using forceps and transferred to a small petri dish containing a 30 mm
160 diameter of filter paper (Whatman No. 1). The samples remained covered when not in use to reduce
161 airborne contamination.
162
163 **Enzymatic digestion of deep-sea macroinvertebrates**
164 Fauna > 4 mm were identified to species level in covered glass petri dishes; individuals of
165 *Ophiomusium lymani* (n = 40), *Hymenaster pellucidus* (n = 19) and *Colus jeffreysianus* (n = 7)
166 were used for microplastics analysis (Figure SI 1).
167 Specimens were removed from the freezer and allowed to defrost while wrapped in
168 aluminium foil for 45 minutes. The length of the central disc (*H. pellucidus* and *O. lymani*), or the
169 shell (*C. jeffreysianus)* were measured with metal dial calipers and the mass of the entire specimen
170 was recorded (Sartorius electronic balance) to the nearest 0.0001 g. Specimens were rinsed
171 thoroughly in a flow of deionised water prior to dissection. Dissections varied slightly between
172 species; for *O. lymani* the central disc was opened in a clean glass petri dish and all tissue was
173 removed from the exoskeleton. For *H. pellucidus* the central disc was opened along with each of
174 the five arms and the tissue was dissected from the body cavity. The shell of *C. jeffreysianus* was
175 crushed by applying pressure and the complete tissue mass was removed. For all species the soft

tissue was weighed using a Sartorius electronic balance and placed in a glass beaker containing 20 ml of 0.3125 % concentration trypsin solution, prepared using Gibco™ trypsin diluted with deionised water (Courtene-Jones et al., 2017). Beakers were covered with glass covers and placed on heated magnetic stirrers set to stir at 250 rpm at 38-42°C and left to digest for 25 minutes.

The resulting mixture was poured through 52 µm mesh gauze before being transferred to a covered glass petri dish. The gauze was thoroughly examined under a Wild M5 dissecting microscope and any potential microplastics were transferred to a small petri dish containing 30 mm diameter filter paper (Whatman No. 1), samples remained covered when not in use to reduce risk of aerial contamination. After all potential microplastics had been transferred to the petri dish it was sealed and labelled for further analysis.

Microplastic identification

The length of each microplastic particle was measured using the ocular scale of a Wild M5 dissecting microscope. Potential microplastics obtained from the water sample and extracted from fauna, along with putative contaminants from the ship and laboratory QA/QC procedures were identified using a Perkin-Elmer Spectrum 100 Fourier Transformation Infrared spectroscope coupled with a universal Attenuated Total Reflectance accessory (ATR-FTIR) equipped with a diamond detector. Each spectra produced was the result from a series of four high resolution scans in the wavelength range 600 - 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Spectra were visualised in OMNIC 9.2.98 (Thermo Fisher Scientific Inc.) with use of the inbuilt libraries to aid identification. The reference library spectra represent clean samples not typically found in the environment. Additional references were generated from plastics from non-typical sources such as beach debris, consumer products and textiles samples to provide more environmentally relevant

199 samples. As well as using these libraries (in-built and user generated), the characteristic functional
200 group signals were examined visually to confirm the identity of the materials being assessed.
201
202 **Scanning Electron Microscope imaging**
203 A sub-sample of the microplastic fibres extracted from deep-sea water (polyester n = 6) and
204 invertebrates (polyester and acrylic n = 8), along with pristine acrylic and polyester fibres obtained
205 from known textile samples (n = 2) were sputter coated with gold-palladium and imaged using a
206 JOEL JSM-6390 Scanning Electron Microscope (SEM) with a 20kV electron accelerating
207 velocity. A series of SEM images, ensuring an overlap of ~80 % between each, were taken of each
208 fibre.
209
210 **Three-dimensional fibre reconstruction and surface area quantification**
211 Three-dimensional reconstructions of the fibre sub-samples imaged with the SEM were rendered
212 using Agisoft Photoscan Professional V1.2.6 photogrammetry software (Agisoft LLC). The
213 software produces high-resolution three-dimensional surface models, from which surface area
214 quantification of complex objects can be achieved as described in Burns et al., (2015) (Summarised
215 in supplementary information). Models were calibrated against objects of known length and by
216 point-to-point measurements, the resolution of the models were 0.01 μm.
217 As fibres visually appeared twisted and flattened, estimates of baseline surface area were
218 calculated for each of the fibres by multiplying length by width, thus assuming particles were
219 analogous to smooth rectangles. These calculations provide an estimation of surface area for each
220 specific sized particle and surface areas achieved with photogrammetric methods are reported as a
221 ratio relative to the baseline.

Statistical analysis

Data was tested for normality using the Shapiro-Wilk normality test and for homogeneity of variance with the Fligner-Killeen test and was found not to meet the criteria for parametric statistics. To assess microplastic abundance, analysis was performed both using the raw microplastic abundance data and after standardising microplastic quantities by the wet weight (w. w.) tissue mass of an individual. Kruskal-Wallis tests were performed on each of these raw and standardised datasets to investigate differences between species, with subsequent posthoc analysis with a Dunn's test. Microplastic surface area data was not normally distributed, therefore a Wilcoxon rank sum test was used to compare baseline to measured surface areas for the deep sea (fauna and water) and pristine fibres.

Generalized linear modeling (GLM) was conducted to relate the response variable (the number of ingested microplastics) to the five factors (organism mass, length, tissue mass, feeding mode and species). Log transformations of organism mass, tissue mass and length were undertaken and the Poisson distribution was used since the response variable was count data. Prior to running the model, collinearity was checked using the Pearson correlation coefficient (indicated by values > 0.6 (Zuur et al., 2010) and the variance inflation factor (VIF; by sequentially removing the variable with the highest value, until all remaining VIFs were below the suggested value of 2 (Zuur et al., 2010)). Those variables found to be collinear (length and weight) were not included in the model, consequently the variables species, feeding mode and tissue weight were retained and considered in relation to the response variable (the number of microplastics). Models with and without interaction effects between all variables (species, feeding mode, tissue weight) were

considered and optimisation was achieved by sequentially removing the least significant variable or interaction term (determined by the highest p-value). Model overdispersion was tested using the dispersion test in the *AER* package and by calculating the residual deviance of the model divided by the degrees of freedom. All statistical analysis was performed in RStudio V 0.99.892 (R Core Team, 2016) with use of the *PMCMR* (Pohlert, 2014), *dunn.test* (Dinno, 2017) *VIF* (Lin, 2015) and *AER* (Kleiber and Zeileis, 2017) libraries.

RESULTS

QA/QC

No microplastics were identified on the filters fitted to the ships water supply. When analysed with ATR-FTIR spectroscopy none of the potential contaminants sampled from the ship (ropes, filters, clothing) or laboratory (sterile consumable packaging, clothing) had spectra which matched that of material found in deep water or invertebrates samples. Laboratory controls yielded similar results; of the 5 fibres found on the atmospheric controls all were identified as cellulose. The number of fibres on TLS varied from a mean of 6.56 ± 2.60 particles prior to laboratory work commencing, to 10.22 ± 4.18 particles after all laboratory work was undertaken. All fibres were blue, red or white and identified as cellulose/cotton with a distinctive ribbon like morphology when examined under the microscope (Figure SI 2).

Identification of microplastics in deep-sea water

ATR-FTIR analysis was performed on 78 potential microplastics obtained from 240 l of deep-sea water; 17 of which were positively identified as synthetic, 28 as cellulose and 33 yielded unclear spectra. This equates to an abundance of 0.0708 synthetic fibres per litre (70.8 particles m^{-3}) of deep-sea water. All microplastics were monofilament fibres of the colours blue (n = 13), red (n = 2) and transparent (n = 2) Five polymers were identified (Figure 2) with polyester comprising the majority of those identified. Sizes of microfibres ranged widely from a minimum of 0.4 mm recorded for Polyethylene Terepthalate (PET) to a maximum of 8.3 mm for an acrylic fibre.

Identification of microplastics in deep-sea invertebrates

A total of 359 potential microplastics were extracted from three benthic macroinvertebrate species (n = 66 individuals), of which 45 were identified as synthetic from their specific transmission spectra, 165 were identified as cellulose and the remaining 149 did not produce usable spectral data. A total of nine polymers were identified, of which acrylic was most abundant (Figure 2). The majority of synthetic material were monofilament fibres (n = 39, 87 %) and the remaining items were fragments (n = 6, 13 %). Items were predominantly blue and red in colour (n = 9, each accounting for 42 % of the total), however black, green, orange, transparent and multi-coloured items were also identified. Mean particle length ranged from a maximum of 6.25 mm recorded for a polyacrylonitrile fibre to a minimum of 0.023 mm for an acrylic fragment, both ingested by *O. lymani* individuals. Overall mean particle length was 1.191 ± 0.0756 mm across all species.

Ingested microplastic quantities varied between individuals and species; considering those individuals from which microplastics were extracted, *O. lymani* ingested the greatest number of polymer types and *H. pellucidus* contained the greatest overall abundance with a mean of 1.582 ± 0.448 SE microplastics g^{-1} w.w. tissue (Table 1). There were significant differences between the

number of microplastics ingested between species (H = 9.7988, df = 2, p = 0.007) explained by a highly significant difference between *O. lymani* and *H. pellucidus* (Dunn's test p = 0.002) and between *H. pellucidus* and *C. jeffreysianus* (Dunn's test p = 0.009) The standardized number of microplastics per gram of tissue also differed significantly between species (H = 7.0629, df = 2, p = 0.0293), again explained by differences between *O. lymani* and *H. pellucidus* (Dunn's test p = 0.010) and between *H. pellucidus* and *C. jeffreysianus* (Dunn's test p = 0.016) (Figure 3).

The final GLM included species and the log of tissue mass as an offset of the response variable, the number of microplastics ingested. No interaction terms were included in the model as these had negligible effects on the results. The model, with a Poisson distribution was found to be slightly overdispersed, thus a quasipoisson distribution was applied to the final model to account for the overdispersion. The GLM results identified that the number of microplastics ingested was related to species and not to the other factors (weight, length or feeding mode). The GLM indicated a significant negative relationship (p = 0.0376) between ingested microplastics offset by tissue mass and *C. jeffreysianus*, indeed this species had a factor of 1.94 less microplastics than other species, however it must be noted that only two individuals ingested microplastics. A positive relationship was found between *O. lymani* and the number of microplastics ingested and the model predicted a factor of 1 times more than in *C. jeffreysianus*, however this result was not significant (p = 0.2949). The number of microplastics ingested by *H. pellucidus* was greater, by a factor of 1.67, for a given tissue weight, this positive relationship was significant at the 0.1 level (p = 0.0845).

Visualisation of microplastics and quantification of surface area

309 Scanning Electron Microscope (SEM) imaging revealed microplastics extracted from deep-sea
310 invertebrates and water to be degraded, with much cracking, pitting, fraying and flaking apparent
311 on the microplastic surface, producing a highly rugose exterior. By comparison, pristine fibres
312 appeared to have a relatively smooth, uniform surface structure (Figure 4 and SI 3). These
313 discrepancies were corroborated by the quantification of fibre surface area. The mean ratio of
314 measured surface area relative to the baseline for pristine fibres was 1.792 ± 0.415 SE. Surface
315 area ratios for fibres extracted from deep-sea samples were more than double that of pristine
316 microplastics; 4.157 ± 0.921 SE and 4.331 ± 1.247 SE for fibres extracted from invertebrates and
317 deep-sea water respectively, this was significantly different from baseline values ($V(15) = 12$, $p = 0.0021$). Baseline surface area values were calculated for a rectangular object as fibres appeared
319 elongated and flattened. Acknowledgment is made that baseline values are only estimates, and
320 fibres are assumed to be analogous to rectangles, however cross-checking these results by
321 computing the ratio of surface area derived from a rectangular object to that of half a cylinder
322 results in $\pi / 2$ which is consistent with the values obtained for the pristine fibres. Therefore, no
323 difference was found if baseline values were calculated for a rectangle or half cylinder.

324

325 **DISCUSSION**

326 The presence of microplastics in deep-sea water and the benthic invertebrate community is clearly
327 demonstrated here, providing further evidence for the widespread distribution of anthropogenic
328 microplastics in the marine environment. Microplastics are heterogeneously distributed in surface
329 waters with concentrations ranging between 0.02 - > 100 particles m^{-3} in the Northeast Atlantic
330 Ocean (reviewed in Lusher, 2015). The present study provides the first quantification of
331 microplastic pollution in deep ocean water and found the concentration to be on the same order as

in surface waters (70.8 particles m^{-3}). While it is possible that microplastics may have been re-suspended from the sediment during sampling, no sediment grains were found on the mesh used to filter the seawater. The CTD was suspended 7 m from the seafloor limiting any potential seabed disturbance and sampling of re-suspended microplastics, giving confidence that the microplastics originated from and are contained within deep water. It is duly noted that this data is based on a single sampling point and thus provides only an initial snapshot of microplastic content in deep water. Many additional bottom water samples are required to more adequately assess the abundance of microplastics present in the deep ocean and provide estimates of deep ocean concentrations; however, this work still represents the first attempt to quantify microplastics in this realm.

Microplastics were identified in all three deep-sea benthic macroinvertebrate species from the phylum Echinodermata and Mollusca examined in this study, with an incidence of ingestion (number of individuals with microplastics / total number of individuals sampled) of 48 % across all species; this, while lower than some coastal invertebrates (Devriese et al., 2015; Welden and Cowie, 2016) is still within the range of incidence values documented for a number of inshore species (Desforges et al., 2015; Foekema et al., 2013; Lusher et al., 2013). Taylor et al., (2016) reported the presence of microplastics in species of deep-sea Echinodermata, Arthropoda and Cnidaria from the Atlantic and Indian Oceans, however singularly sampled species precluded the quantification of ingested microplastics. In the present study, proteolytic enzymes were used to digest soft tissue and extract internalised microplastics without detrimentally impacting the polymers present (Courtene-Jones et al., 2017), allowing for a thorough investigation of ingested microplastics. The quantities enumerated from deep-sea fauna are on the same order as those reported in wild coastal species from a range of taxa (Foekema et al., 2013; Van Cauwenberghe et

al., 2015; Van Cauwenberghe and Janssen, 2014). It is important to note that while visual sorting found potential microplastics in all individuals except one *H. pellucidus* specimen, only a small percentage of particles analysed (12 % for fauna and 22 % for the water sample) were positively identified as synthetic polymers by ATR-FTIR spectrometry. Microplastic quantities presented here are therefore likely to be under-reported, due to the small size of particles and the challenges associated with current analytical methods (Löder and Gerdts, 2015). Technological developments will allow for increased accuracy when investigating micro- and nano-sized plastics ingested by wild fauna.

Microplastic ingestion is demonstrated to vary interspecifically, with significant differences in microplastic abundance between *H. pellucidus* and *O. lymani* and between *H. pellucidus and C. jeffreysianus*. The surface deposit feeder and facultative predator *O. lymani* (Iken et al., 2001; Pearson and Gage, 1984) was identified to contain the greatest number of polymer types, however the predatory sea star *H. pellucidus* (Wagstaff et al., 2014) contained the highest median number of microplastics. Indeed, statistical modelling found *H. pellucidus* contained 1.67 times more microplastics per given tissue mass, while a factor of 1.94 less microplastics were internalised by *C. jeffreysianus* than other species. Feeding mode has previously been shown to influence microplastic ingestion in coastal species (Mizraji et al., 2017; Setälä et al., 2015); however in this study the GLM only identified a relationship between species and ingested microplastic quantities and not with feeding mode. It is not possible to speculate why these species specific differences in microplastic levels occur; it is possible however that the small dataset may have precluded any further relationships from being identified, or there may be some other, as yet unidentified, factors involved in microplastic ingestion and retention in an individual's body.

It is important to note that while this study presents novel findings, small sample sizes of benthic invertebrates, particularly for *C. jeffreysianus* and *H. pellucidus*, and deep ocean water prevents robust estimates of microplastic pollution from being made. Numerous logistical and methodological challenges and costs are associated with sampling the deep sea. This study utilised the full number of samples collected during deep-sea operations in the time available during the DY052 research cruise. Concurrent sediment cores were not within the scope of the research cruise and thus prevented the quantification and subsequent comparison of microplastic levels between all three potential deep-sea 'reservoirs'. Additional sampling at this site and other regions within the Rockall Trough, along with the inclusion of sediment cores would strengthen the dataset and yield cross site and/or temporal replication not available during the DY052 cruise.

Close visual inspection of microplastics extracted from deep-sea samples showed high levels of degradation, including surface cracks, pitting, flaking and fragmentation; producing a mean surface area significantly different to baseline values and in excess of double that of pristine fibres. The duration of microplastics in the environment and the associated degradation has a number of consequences of biological concern. The large surface area to volume ratio, high surface reactivity and small size of particles makes them dynamic in the environment (Mattsson et al., 2015) and can increase toxicity (Jeong et al., 2016). Increased surface area of small degraded particles provides a greater area for the establishment of biofilms which influence sinking velocity (Lobelle and Cunliffe, 2011) and provides increased area for the colonisation of bacteria, including pathogenic species (Kirstein et al., 2016; Zettler et al., 2013). Persistent organic pollutants (POPs) may accumulate in microplastics, and as polymers degrade chemical additives breakdown and leach from the plastic (Engler, 2012), further increasing the toxic effects to organisms.

While factors involved in the horizontal transport of microplastics near the sea surface are relatively well documented (Law et al., 2014), the processes affecting vertical transport of microplastics to the benthos are potentially more complex and not well understood. Sinking velocities are influenced by a number of factors and microplastic behaviour in part is affected by particle size, shape and polymer density (Ballent et al., 2012; Kowalski et al., 2016). The quantity identified in deep-sea water by this study, akin to surface water concentrations indicates processes distributing microplastics throughout the water column. The majority of polymers identified had densities greater than seawater, such as polyester, acrylic and polyamide. Of note, is the presence of positively buoyant polymers, such as polyethylene, which has a specific density of 0.91 - 0.94 g cm^{-3} (Andrady, 2015). In addition to physical properties, microplastic sinking rates are also influenced by interactions with marine organisms, including biofouling (Fazey and Ryan, 2016; Lobelle and Cunliffe, 2011), incorporation into faecal pellets (Cole et al., 2016) and marine aggregates (Long et al., 2015; Ward and Kach, 2009; Wright et al., 2013b; Zhao et al., 2016). These biological processes alter the settling velocity of microplastics by as much as an order of magnitude (Clark et al., 2016; Long et al., 2015). Furthermore species of zooplankton undertake diel vertical migrations (Williamson et al., 2011) which could further redistribute microplastics in the oceans. It cannot be affirmed whether the microplastics isolated from the deep sea in this study arise from the degradation and fragmentation of larger items already located in the deep ocean, or are transported by physical and biological processes through the water column to the seafloor.

Conclusion

This study demonstrates the presence of microplastics in deep-sea benthic fauna and water in the Rockall Trough. Further sampling of water and fauna, along with the addition of sediment cores are necessary to assess ecosystem-wide microplastic pollution in this region and monitor temporal changes. While this study focuses on the Northeast Atlantic Ocean, we hypothesize that microplastics are present throughout the global deep-sea. Further attention and sampling efforts should be directed to the deep oceans globally to establish the prevalence of microplastic pollution in this remote and still largely unstudied ecosystem. The deep sea is vulnerable to a number of anthropogenic pressures (Ahnert and Borowski, 2000; Glover and Smith, 2003; Puig et al., 2012) and now microplastic pollution may be added to these threats, raising concern for ongoing ecosystem functioning. Future steps must work towards understanding the susceptibility and potential impacts of microplastic ingestion by deep-sea species assemblages, and elucidate spatial and temporal vertical transport routes by which microplastics enter and are sequestered in the deep sea.

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Appendix A. Supplementary data

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Figure 1. Map showing the deep-sea sampling locations to the west of the United Kingdom (U.K) and Northern Ireland (N.I). The CTD deep water sampling location (blue circle) and four epibenthic sled trawls (red tracks) are shown around the regular monitoring site 'Gage Station M' (green triangle) to the east of Anton Dohrn (A.D.) Seamount in the Rockall Trough. Area within the dashed line box is shown in more detail in the adjacent panel. Bathymetry is contoured at 500 m intervals from depths of 500 m to 3500 m (MATLAB R2015b using GEBCO_2014 bathymetry data).

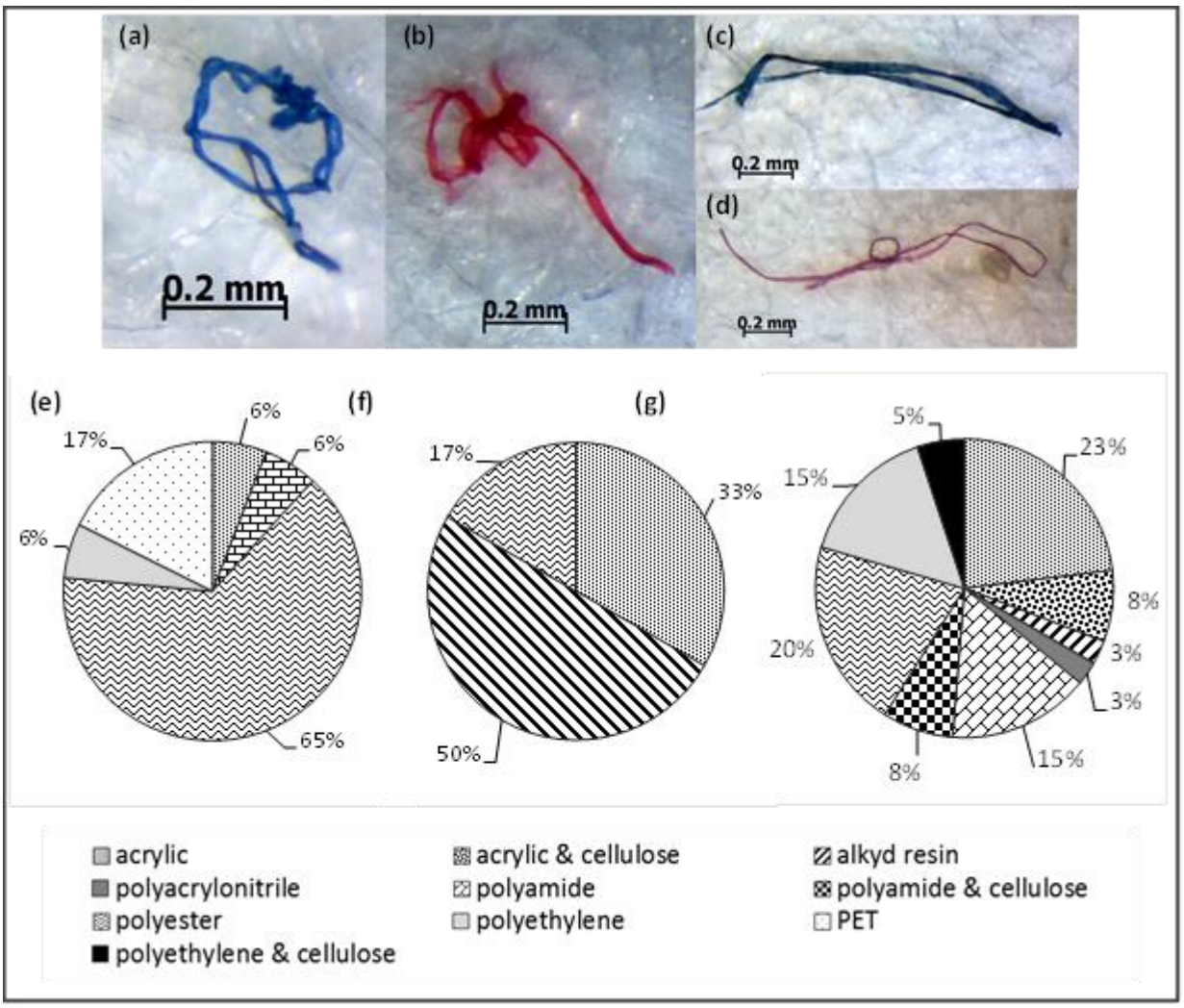

Figure 2. Example microplastic fibres found in (a & b) deep sea water and (c & d) extracted from benthic invertebrates, along with the proportion, as a percentage, of polymer (e) fibres identified in deep-sea water (n = 17); (f) fibres (n = 39) and (g) fragments (n = 6) extracted from deep-sea benthic macroinvertebrates. Differences in relative abundance and polymer diversity are observed between water and invertebrates; polyester is the dominant polymer in deep-sea water, while acrylic accounts for the majority of ingested microplastics by benthic fauna.

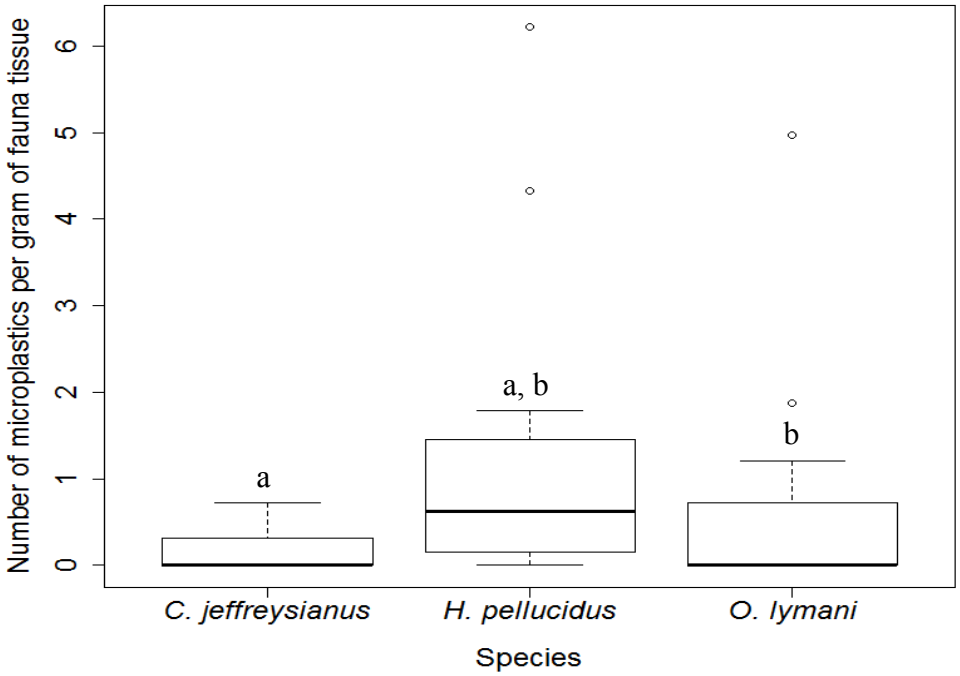

Figure 3. Number of microplastic particles standardized per gram of w.w. tissue ingested by each of the three invertebrate species. Thick black line indicates median value, boxes depict the first and third quartiles and the whiskers show the interquartile range. Outliers are shown by the open points and letters denotes significant differences between species groups.

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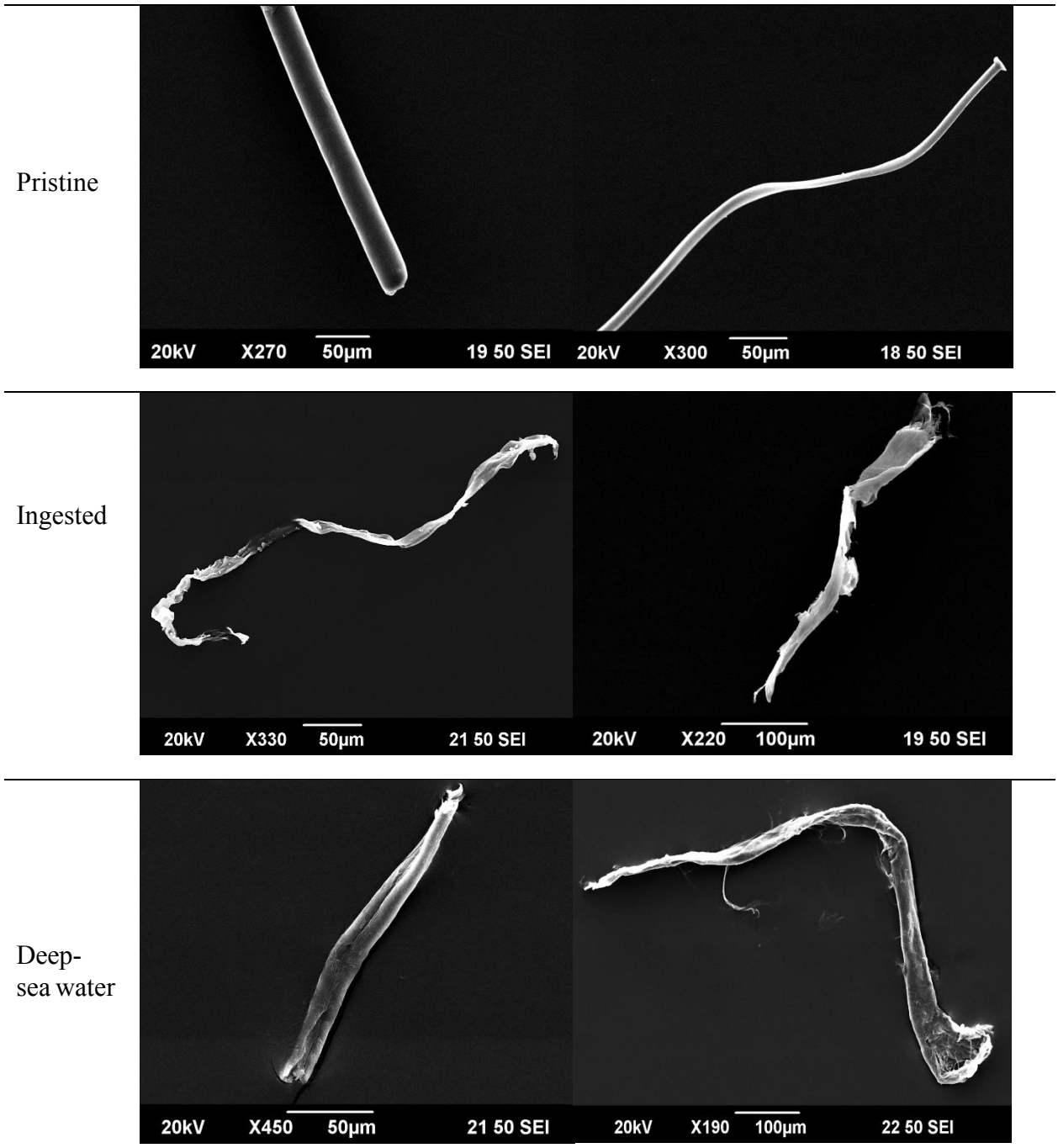

695 **Figure 4**. SEM images of pristine fibres and those isolated from deep-sea water and benthic
696 macroinvertebrates. Fibres from the deep sea show visible surface cracking, pitting, flaking and
697 fragmentation when compared to pristine fibres which are smooth and uniform in appearance.

698

699 **Table 1**. Number of individuals (ind.) sampled and with microplastics internalised, weights and feeding
700 mode for each invertebrate species, along with the mean number of microplastics extracted g^{-1} of wet weight
701 (w. w.) tissue and the total number of polymers ingested.

Species	**No. of ind sampled / No. of ind. with microplastics**	**w.w. tissue mass range (g)**	**Specimen mass range (g)**	**Feeding mode**	**Mean microplastics g^{-1} w.w. tissue**	**No. polymers ingested**
Ophiomusium lymani	40 / 16	0.532 – 2.503	4.296 - 7.050	Deposit feeder/ facultative predator (Iken et al., 2001)	1.153 ± 0.278 SE	9
Hymenaster pellucidus	19 / 14	0.267 - 3.441	0.691 – 12.533	Predator: benthic invertebrates and planktonic fallout (Wagstaff et al., 2014)	1.582 ± 0.448 SE	6
Colus jeffreysianus	7 / 2	1.385 – 3.076	3.129 – 6.980	Predator: burrowing amphipods and bivalves (Kosyan, 2007)	0.678 ± 0.044 SE	2

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